EPA Request for Clarifications of the SAB Draft Peer Review Report on

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

For April 17, 2017 Teleconference

EPA is seeking clarification on some recommendations provided in the Science Advisory Board Chemical Assessment Advisory Committee (SAB-CAAC) draft report, "Review of EPA's Draft Assessment entitled Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (September 2016)." For each area of clarification, text from the draft SAB-CAAC report is provided below, followed by EPA's request for clarification.

Testing of additional endpoints of nervous system effects

Draft Report Language

Cover letter, lines 36–41. However, convulsions in rodents can only provide a limited spectrum of potential human hazard since convulsive or nonconvulsive seizures, epileptiform discharges, reduction in seizure threshold, sub chronic sensitization, and neuronal damages can all be part of the spectrum of RDX's nervous system hazards. Thus, further evaluation or explanation should be provided in the draft assessment for these potential endpoints.

p. 10, lines 30–32. The SAB does not find the reference dose (RfD) derived by EPA for nervous system effects to be scientifically supported and clearly the candidate RfD did not capture all the potential adverse outcomes or their severity.

p. 25, lines 2–7. Endpoints such as convulsions, tremors and aggression are appropriate as part of the spectrum of effects. Additional studies addressing cognitive and behavioral effects of RDX would assist in assessing other endpoints less severe than convulsions. Although there are data from existing animal studies showing changes in behavior, the data are not sufficiently robust to evaluate dose-response relationships, and animal data on cognitive changes are lacking. Given these limitations, additional endpoints are needed to address the complete spectrum of effects.

Clarification

The SAB-CAAC observed that additional endpoints are needed to address the complete spectrum of nervous system effects of RDX; however, these additional endpoints have not been examined in the available toxicity studies of RDX beyond their description and presentation in the draft assessment, and EPA's IRIS Program does not have the regulatory authority to request additional testing. Does the SAB-CAAC mean to suggest that the use of convulsions, given the available data, is not supported for derivation of a reference dose (as implied on page 10, lines 30-32)? It would be helpful if the SAB-CAAC could clarify the above statements in recognition that data to address the characterization of the complete spectrum of nervous system effects are not available. The SAB-CAAC did make this point on page 9 of the draft report stating, "Although there is data from existing animal studies showing changes in behavior, the data are not sufficiently robust to evaluate dose-response relationships, and animal data on cognitive changes is lacking. Therefore, there are no endpoints in existing studies to address the complete spectrum of effects" (lines 23-26). In contrast, the language on page 25, lines 6-7 indicates "additional endpoints are needed to address the complete spectrum of effects," suggesting other endpoints are available to derive a reference dose.

Confidence in the Cholakis et al. (1980) study

Draft Report Language

Cover letter, p. 2, lines 20–21; p 10, lines 32–34. The SAB recommends the draft assessment use the NOAEL from the Cholakis study as the primary basis for the derivation of the RfD for neurotoxicity in addition to the dose-response data of the Crouse study.

Cover letter, p. 3, lines 8–9. Thus, the SAB recommends EPA use a NOAEL of 0.2 mg/kg-day from the Cholakis study as the POD to calculate the RfD.

Clarification

The SAB-CAAC recommends that the teratology study by Cholakis et al. (1980) be used to derive the RDX RfD based on the statement in the study report that "At a dose level of 2.0 mg/kg/day, only one female exhibited convulsions during the period of dosing." The SAB-CAAC describes study quality considerations on p. 57, lines 8–29 in the draft report, noting that the Crouse et al. study was a better study design to detect neurological effects, but placing greater emphasis on the report of a convulsion at 2 mg/kg-day in the Cholakis et al. (1980) study, which is lower than the dose of 8 mg/kg-day observed to cause convulsions in Crouse et al. (2006).

EPA would like to bring to the attention of the SAB-CAAC that convulsions were also observed in one of 24 female rats administered hydroxyurea as a positive control in the Cholakis et al. (1980) teratology study. As noted in Cholakis et al. (1980), "Convulsions were also observed in one female receiving 350 mg/kg/day of hydroxyurea" (report p. 19). Based on a search of the hydroxyurea toxicity literature, no evidence of convulsions in repeat-dose studies in experimental animals was found (see Morton et al., 2015). EPA would like to ask the SAB-CAAC to consider whether the findings of convulsions in a female rat in the hydroxyurea positive control group, along with limitations noted by the SAB-CAAC on p. 31 (lines 35–41) of their report, influences confidence in the use of the Cholakis et al. (1980) study for use as the basis for the RfD.

REF: Morton et al. (2015). Toxicity of hydroxyurea in rats and dogs. Toxicol. Pathol 43: 498-512. http://journals.sagepub.com/doi/pdf/10.1177/0192623314559103

Cholakis et al. (1980) in addition to Crouse et al. (2006) as the basis for the RfD

Draft Report Language

Cover letter, p. 2, lines 20–21. The SAB recommends the assessment use the NOAEL from the Cholakis study as the primary basis for the derivation of a RfD for neurotoxicity in addition to the dose-response data of the Crouse study.

Cover letter, p. 3, lines 8–9. Thus, the SAB recommends EPA use a NOAEL of 0.2 mg/kg-day from the Cholakis study as the POD to calculate the RfD.

p. 25, lines 37–39. …the SAB concludes that it is appropriate to consider the dose-response data reported in the Crouse study as a relevant model. In fact, the Crouse study produced perhaps the best RDX dose-response data available.

p. 33, lines 3–5. Therefore, the draft assessment should utilize the NOAEL and data from Cholakis et al. (1980) as the primary basis for the RfD in combination with the dose-response data of Crouse et al. (2006).

Clarification

EPA is seeking clarification regarding the SAB-CAAC recommendation that EPA base the RfD for nervous system effects on the Cholakis et al. (1980) study (as primary) in addition to the dose-response data from Crouse et al. (2006). On p. 25, the SAB characterizes the Crouse et al. (2006) study as producing perhaps the best RDX dose-response data, but elsewhere (e.g., p. 3 of the cover letter) recommends that the RfD be based on the Cholakis study only. Additionally, the SAB-CAAC expressly rejects quantitatively combining the data from Cholakis et al. (1980) and Crouse et al. (2006) as an option for deriving the RfD (draft report, p. 57–58, lines 43-2). If the SAB-CAAC intends to combine the findings of Crouse et al. (2006) with Cholakis et al. (1980), EPA requests clarification of how that might be accomplished.

Point of departure for nervous system effects

The SAB recommends the use of a benchmark response (BMR) of 5% extra risk (ER) for convulsions in Crouse et al. (2006), based on an argument that the selection of the BMR should be based largely on statistical considerations.

Draft Report Language

p. 27, lines 10–16. "the [BMD Technical Guidance] also points out that '…if one models below the observable range, one needs to be mindful that the degree of uncertainty in the estimates increases. In such cases, the BMD and BMDL can be compared for excessive divergence. In addition, model uncertainty increases below the range of data.' The SAB interprets this guidance (and its own common-sense assessment of dose-response modeling) to imply that the choice of a BMR should primarily be directed by the nature of the data." Related to this, the SAB states in lines 16–18, "The original intent of the BMR and the resulting BMD, as given in the guidance document, is that it should correspond to a response 'near the low end of observable range.'"

Clarification

EPA would like to clarify these aspects of the BMD Technical Guidance (U.S. EPA, 2012). The guidance's recommendation to be "mindful" of the degree of uncertainty is cautionary rather than prescriptive. That is, the guidance statement on comparing the BMD and BMDL for excessive divergence indicates a way to assess the adequacy of the BMD estimation, not an explicit recommendation to select a BMR only "near the low end of the observable range". The guidance also highlights that "[b]ecause different study designs have different dose selections and different sensitivities (i.e., statistical power) to observe adverse effects at various doses, the low end of the observations can correspond to disparate response levels across studies,…." The intent was not to fix the BMR near the low end of the observable range.

Draft Report Language

Cover Letter, p. 1-2, lines 44-1. However, the SAB does not agree with EPA's use of a benchmark response (BMR) of 1% for deriving the lower bound on the benchmark dose (BMDL) as the point of departure (POD) from Crouse et al. (2006). A BMR of 1% would correspond to a response that is a factor of 15 below the lowest observed response data.

Clarification

In the cover letter, as well as the table and accompanying text on page 27, lines 24-35, the SAB-CAAC states that the use of a 1% ER BMR constitutes excessive extrapolation below the LOAEL at 8 mg/kg-day with a 15% response. EPA would like to clarify that in general, selecting the BMR based on extrapolation from the LOAEL ignores the dose-response pattern in the data. EPA notes that the 0% response at the NOAEL of 4 mg/kg-day provides an informative limit on the extrapolation. The BMD estimate for the selected model was 3.02 mg/kg-day, which EPA considers near the low end of the observed range.

Draft Report Language

p. 27-28, lines 37-5. EPA's choice of a BMR of 1% for modeling the Crouse et al. (2006) data is based on the severity of the convulsion endpoint and the proximity (dose-wise) of convulsions to lethality. EPA's rationale for this choice is to provide a sufficient margin of safety between these two endpoints. The SAB agrees that the proximity of these two endpoints is, indeed, a valid source of uncertainty in terms of providing sufficient protection for sensitive human populations. However, the SAB believes that dose-response modeling (including benchmark dose modeling) should focus on the data and what can reasonably be concluded from the data about the dose-response close to the range of the observable data."

Clarification

EPA would like to clarify that the BMD technical guidance states (p. 19), "Selecting a BMR(s) involves making judgments about the statistical and biological characteristics of the dataset and about the applications for which the resulting BMDs/BMDLs will be used," that is, weighing both biological and statistical considerations on a case-by-case basis. In this case, frank effects such as convulsions and are biologically significant. Additionally, the twofold dose spacing and lack of response at 4 mg/kg-day in the Crouse study support a degree of low dose extrapolation. Note that dose spacing was tenfold in the Cholakis study.

The recommendations of the SAB-CAAC regarding the selection and application of a BMR for convulsions were guided by the panel's interpretation of EPA's BMD technical guidance. EPA also notes that the BMD technical guidance was not intended to provide specific guidance in selecting BMRs, because of the need to consider biological issues specific to each dataset. EPA would like to suggest that the SAB-CAAC also take into account that identifying a POD for this RfD (an application not specifically addressed by the BMD technical guidance) needs to address a level of exposure that avoids an appreciable risk of convulsions in sensitive human populations. A POD with a 5% response level for a frank effect is essentially a LOAEL, and would still need adjustment to a lower level to avoid this appreciable risk. It seems constructive to avoid an additional uncertainty factor when a dose-response model can provide some clarity. EPA requests the SAB-CAAC consider whether clarification in the draft report is necessary in light of this information.

Development of a separate organ/system-specific RfD for prostatitis

Draft Report Language

Cover letter, p. 2, lines 23–27. The SAB agrees that kidney and other urogenital system toxicity are a potential human hazard of RDX exposure. However, the SAB disagrees with the selection of suppurative prostatitis as the "surrogate marker" to represent this hazard, and recommends that EPA considers suppurative prostatitis as a separate effect. As such, separate organ/system-specific RfDs should be derived for renal papillary necrosis and the associated renal inflammation and suppurative prostatitis.

p. 11, lines 16–19. The SAB recommends that a separate RfD be derived for renal papillary necrosis and the associated renal inflammation for the kidney and urogenital system and that the male accessory sex glands be designated as a separate organ system, with a separate RfD derived for suppurative prostatitis.

p. 33, lines 33–37. All hazards to the kidney and urogenital system are adequately assessed and described in the draft assessment, with the exception of the description of inflammatory changes in the rat prostate. The description in the draft assessment of these prostatic inflammatory changes should include not only suppurative inflammation, but also chronic inflammation and the variability and uncertainty in the classification of prostatic inflammation.

p. 35, lines 40–42. The SAB recommends suppurative prostatitis not be used as a "surrogate marker" of renal and overall urogenital effects, but instead, be considered as a separate effect (see also Section 3.3.2.5.).

p. 38, lines 32–34. The SAB recommends that separate RfDs be derived for the kidney and urogenital system based on findings of renal papillary necrosis and associated renal inflammation, and for suppurative prostatitis.

p. 40, lines 12–15. The SAB recommends that a separate RfD be derived for renal papillary necrosis and the associated renal inflammation for the kidney and urogenital system and that the male accessory sex glands be designated as a separate organ system with a separate RfD derived for suppurative prostatitis.

<u>Clarification</u>
In reaching a determination to develop an organ/system-specific reference value, EPA first synthesizes and evaluates the available evidence for a given health hazard, and concludes with a hazard determination based on integration of the available human, animal, and mechanistic evidence. Where there is sufficient evidence that RDX exposure is associated with a hazard for a given health outcome, data sets representing that hazard are carried forward for dose-response analysis in Chapter 2.

We understand that the SAB-CAAC's draft advice is to separate prostatitis from other urogenital toxicity and to derive separate RfDs for both the prostate and kidney. In requesting separate RfDs, is the CAAC concluding that the evidence supports prostatitis as a hazard of RDX exposure?

If an organ/system-specific RfD for prostatitis should be derived, EPA is also seeking clarification of whether the analysis should be based on the incidence of suppurative prostatitis only, or all types prostatic inflammation combined (e.g., subchronic, focal; chronic-active; and suppurative).